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(54) **A method of and an apparatus for filling chromatography columns**

(57) The invention relates to a method for the wet filling of chromatography columns in which the sorbent, in suspension in an eluent, is continuously fed into the column and is thickened by filtration under pressure.

Fig. 3

The invention relates to a method for the wet filling of chromatography columns in which the sorbent, in suspension in an eluent, is fed into the column and thickened by filtration under pressure, and also to an apparatus for carrying out this method.

Usually, the chromatography column is closed at one end by an outlet device comprising a filter element and is filled with a generally highly viscous suspension of the sorbent in an eluent through a filling tube fitted at the other end. In order to create a dense column packing, pressure is exerted on the sorbent suspension present in the column and the filling tube, the pressure being generated for instance by movement of a piston (BE 896 865 and DE 24 09 935), by gas pressure (PL 110 372) or particularly by pressure filtration (EP 0 105 583). In the case of the last-mentioned method, the sorbent material is compacted by delivering the eluent through the filling tube and column by means of a pump.

However, where this filling method is concerned, it often happens that the column packings are not sufficiently homogeneous and have only inadequately reproducible separating properties, since at the onset of the filling process, the sorbent material settles, resulting in a differing size distribution of the sorbent over the length of the column. Particularly in the case of methods in which the column filling is thickened by means of a piston or plunger, the sorbent suspension is used at a high density. Also in the case of pressure filtration methods, this is necessary on account of the limited volume of the filling tube. In order to reduce the tendency to sedimentation, it is necessary to use highly viscous dense and often toxic solvents (Balanced Density Method). In the case of prior art packing methods using pressure filtration, any additional mechanical compaction of the column bed is impossible. Thus, the stability of the column bed is limited. Particularly in the case of large columns with finely granular material and considerable packing length, these filling methods cannot usually be employed.

On account of the immense importance of chromatography for analytical and particularly for preparative applications, therefore, there was an urgent need for an improved method for the wet filling of chromatography columns, which does not have the above-mentioned drawbacks found in conventional methods. Furthermore, a compact apparatus for such a method would be desirable, so that the filling and separating means can be combined.

The invention was based on the problem of finding such a method, in which the sorbent, while suspending in an eluent, is fed into the column and thickened by filtration under pressure.

Furthermore, the invention was based on the problem of providing an apparatus for such a method.

This problem can surprisingly be resolved by the provision of the method according to the invention and also the apparatus according to the invention which is designed to carry out this method.

Consequently, the object of the invention is a method for the wet filling of chromatography columns in which the sorbent, suspended in an eluent, is continuously introduced into the column and thickened by filtration under pressure. In this respect, the suspension density of the sorbent suspension is varied by measured admixture of eluent.

Fig. 1 diagrammatically shows an apparatus according to the invention for the wet filling of chromatography columns. Fig. 2 shows a preferred embodiment in which the filling tube has diagrammatically shown peripheral apertures; some parts of the apparatus which are identical and which are already shown in Fig. 1 have not been shown again. Fig. 3 is a detail diagram of filling tube with peripheral apertures, column head and compression plunger.

The method according to the invention is carried out in an apparatus according to Fig. 1, in the following way: in a recipient (1), the sorbent suspension is maintained in suspension by an agitating device (2). In this respect, the density of the sorbent suspension which is defined as a quotient of the amount of sorbent in grammes and the volume of eluent or of suspension in millilitres. Instead of an agitating means (2), similar devices such as for example an ultrasonic homogeniser, can be provided for complete homogenisation of the suspension. The suspension density, as it is fed to the column (10), can be reduced by the admixture of eluent from the receptacles (3). The settings of the two valves (4) and (5) determine the proportion of dilution. The pump (6) conveys the adjusted sorbent suspension to the column (10): either directly or preferably through the filling tube (8) which is mounted on the column (10). In this respect, the working pressure can be measured by means of the pressure gauge (7). The sorbent collects in the

chromatography column (10) above the filtering means (12) at its bottom end. In a preferred embodiment of the method, the eluent is recycled to the vessels (1) and/or (2) via the valves (11) and (9).

According to the method of the invention, any solid sorbents which can be used in chromatography may constitute a filling material. Silica gels in modified and non-modified form, aluminium oxide and polymer carriers may be cited as examples. Since the suspension density can be varied within a wide range, so sorbent materials of all manner of granular size may be used. The granulation is preferably between 1 µm and 500 µm but quite particularly between 2 µm and 300 µm.

As sorbent suspension fluids or eluents, all suitable aqueous and non-aqueous solvents or solvent mixtures may be used, of which only the most important will be quoted here by way of example: methanol, ethanol, propanol, butanol, octanol, cyclo-hexanol, ethylene glycol, diethylene glycol, diethyl ether, dibutyl ether, anisol, dioxane, tetrahydrofurane, mono-, di-, tri-polyethylene glycol ether, acetone, butanone, cyclohexanone, acetic acid ethyl ester, glycol ester, dimethyl formamide, pyridine, N-methyl pyrrolidone, acetonitrile, carbon bisolphide, dimethyl sulphoxide, sulpholane, nitrobenzene, methylene chloride, chloroform, tetrachloromethane, tri-perchloroethylene, ethylene chloride, chlorofluorocarbons, benzines, cyclohexane, methylcyclobexane, decalene, benzene, toluene and/or xylene. In this respect, non-toxic solvents with a high flash point are preferred, the selection of solvents when compared with methods in the state of the art, is only minimally restricted by density and viscosity. Therefore, it is often possible to dispense with using expensive and toxic solvents, since the density of the sorbent suspension is variable and can be adapted to the granular size of the sorbent material. Preferably, the density of the sorbent suspension is between $10^{-3}$ g/ml and 7.5 x $10^{-1}$g/ml.

For conveying the sorbent suspension and eluent, it is possible to use pumps such as for example centrifugal pumps, screw pumps or displacement pumps which are normally used in chemical processing. Preferably, the sorbent suspension is fed into the chromatography column (10) at elevated pressures of up to 900 bars. The rate of

dispensing is preferably adapted to the size of the column to be filled and depends upon the granulation of the filling material used. The dispensing rate indicated as a linear flow rate is preferably between 1 and 200 cm/min but particularly between 10 and 40 cm/min.

A further object of the invention is an apparatus for the wet filling of chromatography columns by pressure filtration of the sorbent suspension and at least containing

- a receptacles for the sorbent suspension (1),
- a dispensing valve (4) for regulating the flow of suspension,
- a receptacles for eluent (3),
- a dispensing valve (5) for regulating the flow of eluent, and
- a pump (6) for feeding the suspension into the chromatography column (1) to be filled and which has filter elements (12).

An apparatus is preferred which additionally contains:

- a filling tube (8) which can be mounted on the chromatography column (10),
- a pressure gauge (7) for measuring the pressure exerted during pressure filtration to thicken the sorbent material,
- further measuring means, e.g. for the through flow rate, and known to a man skilled in the art, and
- dispensing valves (9) and (11) for regulating and guiding the flow of eluent emerging from the column (10).

Particularly preferred and invention *per se* is an apparatus in which the filling tube (8) is constructed in a special manner (see Fig. 3), so that the sorbent suspension can be fed into the filling tube by means of a peripheral ring main (23) though peripherally disposed apertures (24). In a further development of this particularly preferred embodiment:

- a compression plunger (15) with a drive means,
- connecting lines and valves (16) and (20) which make it possible to carry out chromatographic separations without having to remove the column from the filling stand, are also provided.

The column (10) and the filling tube (8) may be rigidly connected to each other or made substantially from one piece.

Further objects of the invention are chromatography columns filled by at least one of the methods according to the invention.

A preferred development of the method according to the invention is shown in Fig. 1, to which reference is made in the ensuing explanation of the method according to the invention.

The sorbent suspension emerging from the sorbent suspension receptacles (1) via the valve (4) can, with the valve (5) closed, be fed into the chromatography column (10) in a density adjusted in the sorbent suspension receptacles (1). The density of the suspension can however be altered in that the valve (5) can be opened and eluent admixed with the sorbent suspension. Further means of mixing two fluid streams in an adjustable mixing ratio are known to a man skilled in the art; for example, each of the two receptacles (1) and (2) may be provided with its own delivery pump for feeding its respective fluid into the filling tube (8) or directly into the column (10), possibly via a mixing chamber. Particularly prior to entry into the mixing zone, suspension and eluent are fed through fine-mesh wire screens or similar obstructions, so producing an additional turbulence and a high degree of homogeneity in the resulting sorbent suspension.

The sorbent suspension density adjusted in the receptacle (1) can be varied within a wide range by the admixture of eluent. In this respect, the admixed eluent may be identical or different from that used in the receptacle (1).

Before entering the filling tube (8), the sorbent suspension is preferably at a density between $10^{-3}$ g/ml and $7.5 \times 10^{-1}$ g/ml but particularly between $1 \times 10^{-2}$ g/ml and $2.5 \times 10^{-1}$ g/ml. By steadily increased in the amount of admixed eluent, however, it is possible to adjust gradients of sorbent suspension density which may be advantageous for many separation applications.

The regulating processes needed to adjust the desired sorbent suspension densities are preferably automated. In this respect, the eluent or sorbent suspension volume delivered in a given unit of time is measured and compared with a preset desired value and any difference which exists is counteracted by regulating the valves (4) and (5).

By the continuous and automatable filling process, more homogeneous packing densities are achieved than with conventional filling methods and a quantity of column packings is to a great extent reproducible.

In contract to conventional filling methods, the solvent, after passing through the column (10) via the valves (11) and (9), can be recycled to the eluent receptacle (3) or sorbent suspension receptacle (1). Thus, the consumption of solvent can be considerably reduced.

After the sorbent material has been thickened, the chromatography column (10) is filled at a predetermined pressure which is dependent upon the acceptable maximum pressure of the column (10) to be filled and the output capacity of the pump (6) which is used. Usually, pressures up to about 900 bars are used.

In Fig. 2, to which reference is made during the following explanation of particularly preferred methods according to the invention, such a particularly preferred apparatus according to the invention is illustrated. Fig. 3 shows corresponding details of the upper column head with filling tube and compression plunger. In this apparatus, the filling tube (8) is of a special design: it has a peripheral channel (23) for the sorbent , from which apertures (24) lead out into the interior of the filling ring. Furthermore, as can be seen by way of example in Fig. 3, the bottom end of the filling tube is shaped in a way which makes it possible to connect it to the column in such a way that there is on the inside a smooth transition between both parts. For the rest, after the points of intersection (13) and (14), there are the basic component elements, as already known from Fig. 1, receptacle (1) for sorbent, with an agitating means (2) and valve (4), as well as receptacle (3) for eluent with valve (5) as well as pump (6) and valve (9).

Hereinafter, the filling of a column by using the particularly preferred method will be disclosed: the compression plunger (15) is moved into the position shown in Fig. 3. From receptacle (3), the column is first filled with eluent; while this is happening, the valve (18) is open and the valve (19) is closed. Then, the sorbent suspension is introduced via the filling tube; while this is happening, the valve (18) is closed while the valve (19) is open, so that the eluent can flow out of the suspension and back through the bottom filter element (12) and into the receptacle (1) and/or (3). Once filling with the sorbent is

completed, firstly eluent is pumped from the receptacle (30 and through the column and then the compression plunger is lowered onto the sorbent packing and the packing is stabilised by additional mechanical pressure. The compression plunger remains on the packing.

Should it turn out that the desired length of sorbent bed is still not reached, the compression plunger can be raised again and further sorbent suspension added.

By virtue of the compact design of the arrangement according to the invention, the filled column can, while in the filled state, be used for chromatographic separations. Cumbersome handling and moving of columns which may be very heavy are therefore avoided. For this purpose, the valves (16) and (20) are so adjusted that there is a communication (22) for introducing samples and to the supply of eluent and so that there is furthermore a connection (21) to the eluate collecting device. Means of introducing samples, eluent admixture and eluate collection are known to a man skilled in the art.

Furthermore, this particularly preferred method has the advantage that the columns can be filled in a closed apparatus and that consequently, for example the workplace exposure to solvent vapours and sorbent dusts is considerably reduced.

As a result of the method according to the invention, chromatography columns in the apparatus according to the invention are filled and have a more homogeneous packing density and a greater reproducibility of the column packing quality than columns which are filled by conventional methods. Since the normal suspension density can be varied within a wide range, it becomes possible to use sorbent materials of the most widely diverse granulations for filling purposes. Since the suspension is added continuously, also large chromatography columns can be filled with very long packings. Therefore, a substantial economic importance accrues to the method and apparatus according to the invention.

In addition to the preferred embodiments of the invention which were described at the outset, equivalent solutions will be readily accessible to a man skilled in the art on a basis of the teaching contained in this Application.

The following examples are intended to illustrate but not to restrict the invention.

**Examples**

Example 1

In an apparatus according to Fig. 1, a column 600 mm long and with an inside diameter of 200 mm is filled, LiChroprep® Si 60 with a granular size of 25 µm to 40 µm (a product sold by E. Merck, Darmstadt, Germany) is used as the sorbent and n-propanol is used as the eluent. The suspension density is 0.1 g/ml and the dispensing rate is 8.5 litres suspension/min (corresponding to a linear flow rate of 25 cm/min). The column is filled at a pressure of not more than 150 bars. After flowing through the column, the eluent is fed back to the eluent stock through valves.

Example 2

In an apparatus according to Fig. 2, a column 600 mm long and having an inside diameter of 200 mm is filled, Lichroprep® Si 60 with a granulation of 25 µm to 40 µm (a product sold by E. Merck, Darmstadt, German) is used as the sorbent and n-propanol is used as the eluent. The suspension density if 0.1 g/ml and the dispensing rate is 8.5 litres suspension/min. The column is filled at a pressure or not more than 150 bars. After flowing through the column, the eluent is fed back to the eluent stock through valves.

Once the column has been filled with all the sorbent, firstly 50 l of eluent are pumped out of the stock (3) and through the column, after which the compression plunger is lowered onto the sorbent packing and the packing stabilised by additional mechanical pressure at 150 bars. The compression plunger remains on the packing.

Example 3

In an apparatus according to Fig. 2, a column 600 mm long and having an inside diameter of 200 mm is filled, the sorbent used being Lichroprep® Si 60 with a granulation of 25 µm to 40 µm (a product sold by E. Merck, Darmstadt, Germany) while the eluent used

is n-propanol. Firstly, from the stock (1), 20 litres of a concentrated suspension (0.45 g/ml) is filled into the column. Then, upon further filling, eluent from stock (3) is added so that during flow into the column, the suspension density is 0.1 g/ml. The dispensing rate at this stage is 8.5 litres suspension/min. The column is filled at a pressure of not more than 150 bars. After flowing through the column, the eluent is fed back to the eluent stock through valves.

Once the column has been filled with all the sorbent, firstly 50 litres of eluent are pumped through the column from the stock (3) and then the compression plunger is lowered onto the sorbent packing and the packing is stabilised by additional mechanical pressure at 150 bars. The compression plunger remains on the packing.

**Patent claims**

1. A method of filling chromatography columns in which the sorbent, in suspension in an eluent, is fed into the column and thickened by filtration under pressure, characterised in that the sorbent suspension is fed continuously into the column and in that the viscosity and density of the sorbent suspension can be varied by adding measured quantities of eluent.

2. A method according to claim 1, characterised in that the suspension is supplied through one or a plurality of peripheral apertures in the filling tube.

3. An apparatus for the wet filling of chromatography columns by the pressure filtration of a sorbent suspension, at least containing respective receptacles with outlet valves (4) and (5) for sorbent suspension (1) and for eluting agent (3), a mixing device (2), a pump (6) for conveying the sorbent suspension, and also the column (10) which is to be filled.

4. An apparatus according to claim 3, characterised in that the aperture or apertures for the feeding of sorbent suspension is/are provided on the periphery of the filling tube.

5. A chromatography column filled by a method according to claim 1 or 2.

Three sheets of drawings